Comparative Study of In Vitro Caries-Like Lesions and Natural Caries Lesions at Crown Margins

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Purpose: **Secondary caries at crown margins and the influence of preparation techniques are major clinical problems. It was therefore the aim of this study to compare natural caries lesions at crown margins with experimental caries lesions.**

Materials and Methods: **Five extracted caries-free human molars were restored with gold cast crowns and afterwards covered with wax leaving a 3 × 3 mm window at the crown margin. These teeth were then incubated in acidified gel (pH 4.7) for 50 days. After incubation these teeth and five other crowned extracted teeth exhibiting natural caries lesions were embedded in Technovit 9100. Serial sections with a thickness of 80 μm were cut through the lesions and investigated with polarized light microscopy (PLM) and scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) analysis for quantitative element analysis of Ca, P, and C. The results of the quantitative element analysis were statistically evaluated using the nonparametric ANOVA test for repeated measurements.**

Results: **PLM of the experimental lesions showed homogeneous lesions with no transparent zone, or dead tracts. The natural caries lesions exhibited a demineralized zone, translucent zone, and dead tracts. Quantitative element analysis showed a statistically significant difference ($p < 0.01$) of Ca, P, and C between sound dentin and demineralized dentin in natural and experimental caries lesions.**

Conclusion: **The experimental model reproduces the demineralization pattern of secondary caries but does not simulate the vital dentin reactions of peritubular and intratubular mineralization.**

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INDEX WORDS: caries, crown margins, in vitro lesions, natural lesions

SECONDARY CARIES at crown margins is a major clinical problem, reducing the lifetime of dental restorations, altering dental hard tissue, and endangering the survival of the tooth. Several factors have been discussed as reasons for the development of secondary caries at crown margins. The most important seems to be the quality and morphology of the crown margin and its integrity to dental hard tissue.[1-4] Large gaps at the crown margin allow accumulation of plaque, which fosters gingivitis and hence, caries development.[5] Another crucial factor is gingival attachment to the restoration[6] and gingival recessions, which develop in 71% of crown margins.[7] It has been shown that secondary caries occurs more often at subgingival margins and is dependent on the preparation technique.[8]

Preparation technique not only determines marginal integrity of the restoration, but also influences the reactions of the residual dentin and the pulp.[9] In a prospective study on 11 teeth with marginal periodontitis, the influence of preparation techniques on the degenerated pulp was studied. Histological sections were evaluated, and typical morphological changes were 3D-reconstructed. The histological findings documented typical endodontal changes due to marginal periodontitis[10] superimposed upon by reactions related to full crown preparation procedures. Both feather-edge preparations and shoulder preparations exhibited similar endodontic findings. The remaining dentin thickness was the

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Accepted May 15, 2006.

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1059-941X/07

doi: 10.1111/j.1532-849X.2007.00220.x

most important factor influencing the intensity of degenerative changes.

A retrospective histological study on 104 teeth 3 to 28 years after fixed prosthodontic treatment revealed typical pathological reactions of the dental pulp adjacent to the prepared margin.[11] Even without additional trauma caused by caries[12] or dental treatment,[13] necrotic degeneration occurred years after crown preparation, increasing when the remaining dentin thickness was less than 2 mm, confirming the results of the prospective study.[9] Additional degenerative changes, including irregular irritation of dentin, inflammation, and even pulp necrosis, were enhanced by the already degenerated pulp or because of the localization of the caries lesion at the crown margin.

Therefore, diagnostic criteria correlated to clinical characteristics of caries progression are necessary to evaluate lesions at the crown margin.[14] Clinical as well as radiographic techniques exhibit specific diagnostic limitations[9] due to the often interproximal or subgingival location of the lesion or due to the masking of the lesion by the radio-opaque crown.

Although the morphology of secondary caries lesions seems to follow the same patterns as primary caries lesions regarding development of their zone profiles, their progression into dentin seems to be different with regard to the penetration at the border between the crown margin and dentin.[15] Secondary caries lesions progress along the crown margin into dentin, perhaps due to acid diffusion along the marginal gap. Therefore, the development and progression of secondary caries lesions at crown margins may also depend upon the quality of luting cement used for fixation of the restoration.

Because of the numerous open questions regarding the pathogenesis and progression of secondary caries lesions at crown margins, further experimental studies are needed to answer some of these questions. Reliable experimental study protocols are needed for further studies. It was therefore the aim of this investigation to evaluate experimental caries lesions at crown margins by means of polarized light microscopy (PLM) and scanning electron microscopy (SEM) and to compare experimentally induced caries-like lesions with natural secondary caries lesions at crown margins.

Materials and Methods

Five totally impacted caries-free human molars and five molars with natural caries lesions at crown margins were stored in 0.9% NaCl containing 0.1% Thymol. The crowns of the caries-free teeth were prepared with feather-edge preparations, and gold crowns were fixed on the teeth using zinc-phosphate cement (Harvard, Richter and Hoffmann, Berlin, Germany). The prepared mesial margin in all teeth was within the root cementum and distally within the enamel. After fixation of the gold crowns, the teeth were covered with wax, leaving a 3 × 3 mm window on the mesial and distal sides, and demineralized in acidified gel at pH 4.7 for 50 days. The teeth with experimental caries-like lesions and natural caries lesions were then embedded in Technovit 9100 (Heraeus Kulzer, Wehrheim, Germany), and serial sections of 80 μm thickness were cut through the lesions with a saw microtome (Leica, Bensheim, Germany). The sections were examined with PLM (Leica DMRB, Wetzlar, Germany). Three sections of each tooth were then coated with carbon and examined with a scanning electron microscope (Philips XL 30 FEG, Philips, Eindhoven, The Netherlands) at 20 kV using the backscattered electron detector to compare the micromorphologic PLM features with the SEM appearance of the different dentin areas. The dentin areas to be investigated by energy dispersive X-ray (EDX) spot measurements were divided into sound dentin (about 0.5 mm distance to the pulp), translucent zone, and demineralizing dentin. All spots for the measurements were placed in areas of intertubular dentin. In each dentin area, three spot measurements (spot size 2 nm) were made, resulting in a total of nine measuring points per area for each tooth. Element content in weight percentage of Ca, P, and C was measured with EDX analysis with an S-UTW detector (EDAX INC, Mahwah, NJ). The count rate of the EDX detector was between 1800 and 2000 counts per second with a dead time of 30%. Measuring time was 30 seconds with a resolution of 135.8 eV and an amplification time of 50 μs. Line scans through the lesions were made at 256 points with a dwell time of 1000 ms and amplification time of 100 ms. The values of the spot measurements were statistically evaluated using the nonparametric ANOVA test for repeated measurements. As three calculations were made on the same set of data, the Bonferroni correction for $p = 0.05$ was $p = 0.016$.

Results

Morphology of Lesions

PLM of the natural caries lesions showed demineralized dentin, the translucent zone underneath

Figure 1. Polarized light micrograph of a natural secondary caries lesion at a crown margin. Softened dentin on the surface, demineralized dentin (yellowish-brown), the translucent hypermineralized dentin (green), and dead tracts are distinguishable. There is a slight undermining of the crown margin, above.

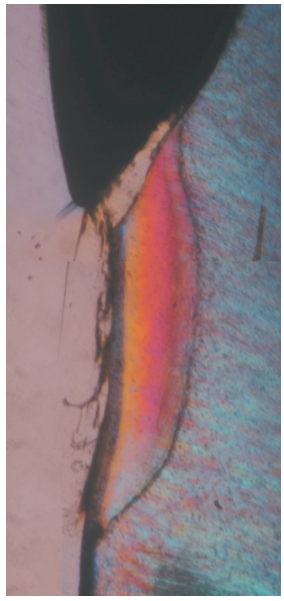

Figure 2. Polarized light micrograph of an experimental caries lesion at a crown margin showing a half-moon shape of the lesion with no translucent zone.

demineralized dentin, and sound dentin (Fig 1). These features were also seen with SEM. The experimental lesions all showed a half-moon-like shape with a demineralized zone but no translucent zone (Fig 2). Higher magnification of the translucent zone in the SEM showed intratubular dentin in natural lesions (Fig 3), whereas the dentin tubules in the experimental lesions were empty underneath the demineralized zone. All natural caries lesions undermined the crown margin, whereas in the experimental lesions 43.3% were at the border of the crown margin and 56.6% undermined the crown margin.

Quantitative Element Analysis

Element analysis revealed different Ca, P, and C content in the different zones of the lesions in natural and experimental lesions. The lowest Ca and P content was measured within the demineralized zone of the natural lesions (Ca: 20.1 ± 8.08 wt%; P: 9.98 ± 3.41 wt%). A slightly higher Ca and P content was found in the hypermineralized zone in natural lesions with a Ca content of 25.57 ± 4.68 wt%; the highest values were determined in sound dentin with a Ca content of 30.78 ± 5.44 wt% and a P content of 16.09 ± 2.09 wt%. In the experimental lesions, the Ca content of demineralized dentin was 16.08 ± 2.09 wt%, and the P content was 12.05 ± 4.68 wt%. The C content showed reciprocal values to the Ca and P content in the different zones (Figs 4 and 5). The results of the quantitative element analysis for natural and artificial lesions are summarized in Table 1.

Statistical evaluation of the spot measurement showed significant differences in the element

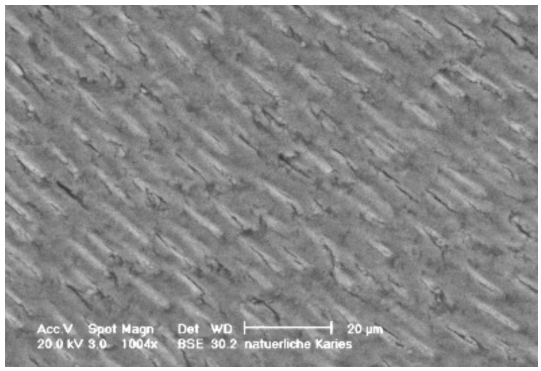

Figure 3. Scanning electron micrograph of intratubular dentin in the hypermineralized zone of a natural secondary caries lesion.

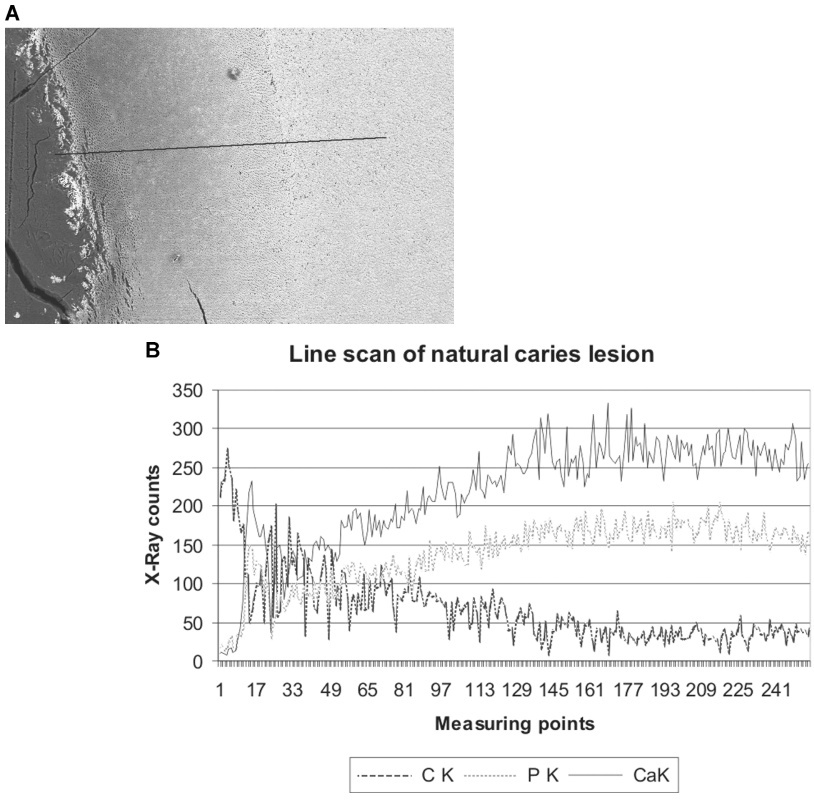

Figure 4. (*A*) Scanning electron micrograph of a natural marginal secondary caries lesion with the line indicating the direction of the line scan. (*B*) Line scan of the elements Ca, P, and C in a natural marginal caries lesion showing the continuous demineralization of dentin from sound dentin toward the surface.

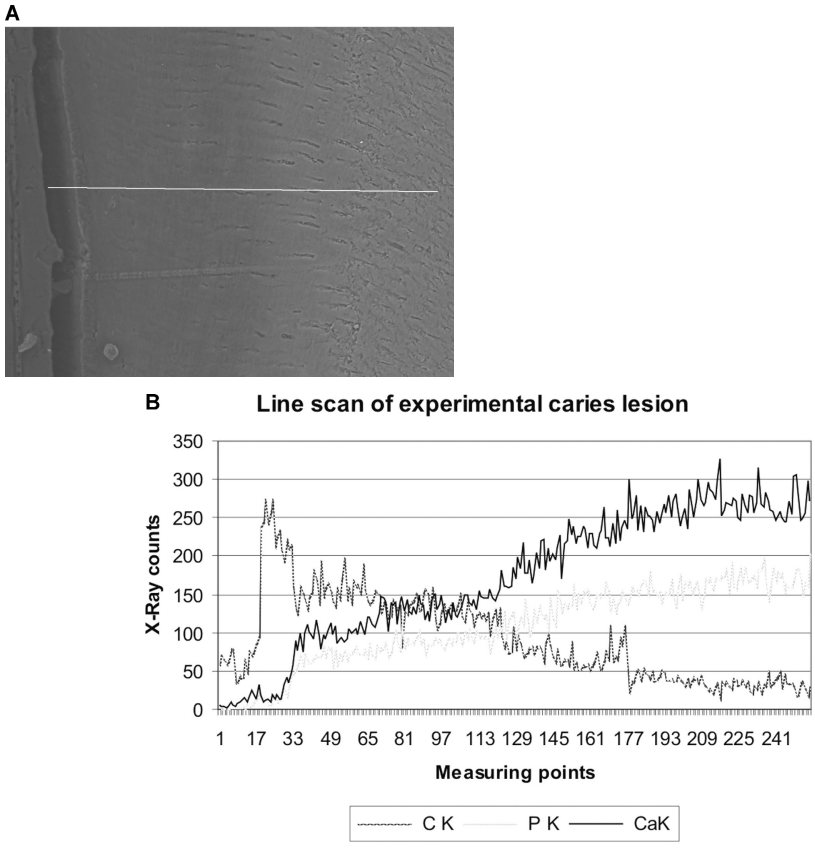

Figure 5. (*A*) Scanning electron micrograph of an experimental marginal caries lesion with the line indicating the direction of the line scan. (*B*) Line scan of the elements Ca, P, and C in an experimental marginal caries lesion showing the continuous demineralization of dentin from sound dentin toward the surface.

Table 1. Element Content and Ca:P Ratio in the Different Zones of Natural and Experimental Marginal Carious Lesions

Lesion Zone	*Ca*		*P*		*C*		*Ca/P*	
	Natural	*Artificial*	*Natural*	*Artificial*	*Natural*	*Artificial*	*Natural*	*Artificial*
Demineralized dentin	20.10 ± 8.08	16.08 ± 2.09	9.98 ± 3.41	12.05 ± 4.68	41.94 ± 12.71	46.59 ± 16.01	2.01	1.98
Hypermineralized dentin	25.57 ± 4.68	Not present	12.05 ± 4.68	Not present	36.44 ± 14.0	Not present	2.12	Not available
Sound dentin	30.78 ± 5.44	34.81 ± 4.1	16.09 ± 2.09	17.68 ± 1.42	20.25 ± 5.53	18.19 ± 3.27	1.91	1.97

Table 2. Statistical Comparison of the Element Content in Different Lesion Zones of Natural Caries Lesions

	Sound Dentin	*Demineralized Dentin*	*Hypermineralized Dentin*
Ca	$p < 0.001$	$p = 0.045$	$p = 0.015$
P	$p < 0.001$	$p = 0.043$	$p < 0.001$
C	$p < 0.001$	$p = 0.023$	$p < 0.001$

content for Ca, P, and C between sound dentin and demineralized dentin in natural and experimental caries lesions. In natural lesions, the difference between sound dentin and hypermineralized dentin was also statistically significant. The difference between hypermineralized dentin and demineralized dentin was statistically not different. All statistical results are summarized in Table 2.

Discussion

The zone profile of the natural caries lesions at crown margins followed the same patterns as in natural dentin lesions with a superficial demineralized zone, a hypermineralized translucent zone, and dead tracts.[16-18] Intratubular dentin apposition by odontoblasts is a natural defense mechanism to prevent further bacterial invasion and dentin destruction.[19-21] However, occlusion of dentin tubules by intratubular dentin is never complete, and at least 25% of the dentin tubules remain open; the intertubular dentin of the translucent zone also appears to be demineralized to some extent.[16] Similar results have been obtained in this investigation, as the mineral content of the hypermineralized dentin was similar to that of demineralized dentin. The absence of the hypermineralized translucent zone in experimental carious lesions is due to the absence of vital odontoblasts, but may be neglected in caries experiments because of the obvious demineralization of intertubular dentin in natural caries lesions.[16-18,22] The demineralization pattern of the experimental lesions was the same as in natural lesions with a significant difference in the Ca and P

content between demineralized dentin and sound dentin.

The extension of the experimental lesions undermined the marginal edges of the crown in 56.6% of the cases. This indicates that the demineralizing acid diffused underneath the crown, dissolving the luting cementum. In natural lesions, the progression of the carious lesion followed the dentin tubules toward the pulp and only undermined the crown in very large lesions.

It has been postulated that the marginal gap between the restoration and dentin is the main reason for secondary caries development[1,4,23] despite the fact that even with highly sophisticated technology there is always a marginal gap. As dentin tubules at the edges of the preparation are not closed and bacteria can easily penetrate into the tubules, the incidence of secondary caries should be rather high and the lifespan of the restorations short. However, Leempoel[24] demonstrated that after 12 years, 87% of the crowns and bridges were still in situ. Assuming that there is always a marginal gap at restorations, the gap itself cannot be the reason for the development of secondary caries. The oral cavity is a complex ecological system in which salivary composition and microbial environment play a very important role in demineralization and remineralization processes on the tooth surface. Therefore, development of secondary caries at margins of restorations is of multifactorial genesis including salivary composition, bacterial colonization, age, dental hygiene, fluoride bioavailability, and quality of the restoration.[25] More experimental and clinical studies are necessary to develop suitable prevention regimes for secondary caries expression and to understand the complex interaction between the restoration, the oral environment, and the tooth surface.

Conclusions

It can be concluded that within the limits of these in vitro experiments, this chemical demineralization model mimics natural carious lesions in dentin and is suitable for the investigation of certain questions regarding secondary caries lesions at crown margins. It has already been used for the correlation of clinical and radiographic findings at crown margins[9] and may further be suitable for studies of the dissolution of cementing materials in connection with secondary caries progression.

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